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ORIGINAL PAPER

Baseline glucose level is an individual trait that is negatively associated with lifespan and increases due to adverse environmental conditions during development and adulthood

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Abstract

High baseline glucose levels are associated with pathologies and shorter lifespan in humans, but little is known about causes and consequences of individual variation in glucose levels in other species. We tested to what extent baseline blood glucose level is a repeatable trait in adult zebra finches, and whether glucose levels were associated with age, manipulated environmental conditions during development (rearing brood size) and adulthood (foraging cost), and lifespan. We found that: (1) repeatability of glucose levels was 30%, both within and between years. (2) Having been reared in a large brood and living with higher foraging costs as adult were independently associated with higher glucose levels. Furthermore, the finding that baseline glucose was low when ambient temperature was high, and foraging costs were low, indicates that glucose is regulated at a lower level when energy turnover is low. (3) Survival probability decreased with increasing baseline glucose. We conclude that baseline glucose is an individual trait negatively associated with survival, and increases due to adverse environmental conditions during development (rearing brood size) and adulthood (foraging cost). Blood glucose may be, therefore, part of the physiological processes linking environmental conditions to lifespan.

Keywords Baseline glucose · Early-life environment · Foraging cost · Repeatability · Survival · Thrifty phenotype hypothesis · *Taeniopygia guttata*

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Introduction

Glucose regulation is a key aspect of homeostasis and difficulties maintaining such balance are associated with detrimental effects. For example, experimentally induced low glucose causes death within 3 to 4 days in chickens *Gallus gallus domesticus* (Akiba et al. 1999). Interestingly, birds maintain nearly twofold higher glucose levels than mammals of similar body mass apparently without experiencing detrimental effects (Braun and Sweazea 2008). However, increased glycosylated hemoglobin, resulted from holding chronically high blood glucose, impaired survival in collared flycatchers *Ficedula albicollis* (Récapet et al. 2016), but was associated with earlier laying dates and larger clutches in the same species (Andersson and Gustafsson 1995), advocating that glucose metabolism and associated physiology (e.g. glucocorticoid levels; Jimeno et al. 2017a) may modulate life-history trade-offs. Accordingly, blood glucose concentration is not fixed, but modulated depending on life-history stage and environmental factors suggesting an adaptive potential for such variation (Schradin et al. 2015). For example,

glucose levels are higher in birds during demanding stages such as parental care (Gayathri et al. 2004) and migratory periods (Bairlein 1983), and increase with age (Prinzinger and Misovic 2010). The latter suggests that despite variating with life stage and environmental conditions, baseline glucose may be an individual characteristic, but we are not aware of repeatability estimates of glucose levels in birds. Thus, blood glucose levels are a complex trait that is likely to be target of selection, but especially in birds there is little information about causes and consequences of its individual variation.

Adverse developmental conditions have long-term effects on offspring fitness prospects in many species, including both birds (Gustafsson et al. 1995; de; Kogel et al. 1997; Lindström 1999; van de Pol et al. 2006; Reid et al. 2010; Boonekamp et al. 2014), and mammals (Ozanne and Hales 2004; Kerr et al. 2007; Plard et al. 2015). The mechanism(s) mediating these effects are not well understood, particularly in birds. However, it is evident that direct or indirect nutritional restriction (i.e. mediated through sibling competition) has long-term effects on avian energy metabolism. For example, restrictive nutrient conditions during development associate with higher energy metabolism in adult birds (Verhulst et al. 2006; Criscuolo et al. 2008; Schmidt et al. 2012). However, whether adverse developmental conditions have long-term effects on glucose homeostasis in adult birds has to our best knowledge not been investigated.

Food availability is a key ecological variable that can have major consequences for reproduction and survival (e.g. Schubert et al. 2009; Verhulst et al. 2004). Food availability also affects blood glucose levels. For example, partial food deprivation in captive birds initially results in lower glucose levels, while prolonged starvation and refeeding both result in higher glucose levels (Savory 1987; Lone and Akhtar 1988; Alonso-Alvarez and Ferrer 2001; Rodríguez et al. 2005; Khalilieh et al. 2012). However, in natural conditions, food availability usually varies through differences in the effort required per item of food obtained (i.e., foraging cost). Such variation may have effects on physiology that are very different from the response to food-deprivation and thus little is known about the effect of naturalistic variation in food availability on glucose levels.

In this study, we used adult zebra finches *Taeniopygia guttata* living in outdoor aviaries, to investigate variation in baseline blood glucose levels (from here on "baseline glucose"; see methods for operational definition). The birds were housed in single sex aviaries, and hence non-breeding. We evaluated whether baseline glucose was: (1) repeatable within individuals over weeks and years, (2) affected by variation in physical ambient conditions (i.e. temperature, day length), (3) related to mass and age, (4) affected by the developmental environment (either large or small rearing brood size), foraging costs (either low or high foraging cost) and their interaction, and (5) associated with survival probability. Considering previous findings in other species (Bairlein 1983; Remage-Healey and Romero 2000; Schradin et al. 2015), we predicted baseline glucose to be a repeatable trait, with higher levels on shorter and colder days, and positively associated with age and mass. In this species, including our study population, being raised in large broods impairs growth and results in a smaller body size at adulthood and lower survival (De Kogel 1997; Tschirren et al. 2009; Briga et al. 2017). Similarly, dealing with high foraging costs in adulthood deteriorates survival prospects (Briga et al. 2017). Thus, growing up in a large broods and being exposed to high foraging costs both constitute adverse environments, and the combination of harsh developmental (large brood size) and adult (high foraging cost) environments impact survival prospects (Briga et al. 2017). Based on this finding, and the reported link between glycosylated hemoglobin and survival in collared flycatchers (Récapet et al. 2016), we predicted birds experiencing adverse conditions, either during development or in adulthood, to have higher baseline glucose.

Methods

Birds and Housing

Birds were bred in the zebra finch breeding colony of the University of Groningen, in 'breeding cages' of 80 × 40 × 40 cm with a nest-box and nesting material (hay). Each breeding cage contained a single randomly formed pair, with unrestricted access to cuttlebone, water and sand. Breeding pairs were food supplemented (egg food, Bogena, Hedel, the Netherlands) regularly until the chicks hatched. Nests were checked daily around the expected date of hatching. Brood size and adult foraging effort were manipulated, for all birds in the study, using a fully factorial design described below (see "Experimental treatments").

Experimental treatments

Developmental conditions of all birds entered in the experiment were manipulated by cross-fostering all chicks to create broods that were either small (two chicks) or large (six chicks). Cross-fostering took place when the oldest chick of a birth nest was 4–5 days old. Resulting brood sizes were within the range observed in wild zebra finches (Zann 1996). After nutritional independence, from the age of 35 days, young were housed in larger ($L \times W \times H$: 153 × 76 × 110 cm) indoor cages with up to 40 other young of the same sex, and two male and two female adults (tutors for sexual imprinting) until the beginning of the adult treatment. They remained in

this setting until they were approximately 120 days of age (when sexually mature).

From approximately 120 days of age, birds were housed in eight single-sex outdoor aviaries (L × H × W: 310 × 210 × 150 cm) located in Groningen, the Netherlands (53° 13′ 0″N / 6° 33′ 0″E). Individual birds were identified using numbered leg rings. Each aviary contained 15–25 birds, and aviaries were regularly restocked with similarly reared birds to maintain numbers approximately constant. At the time of glucose measurements subjects were 0.4–8.4 years old (mean ± SEM 3.3 ± 0.11 years). All birds were provided with a tropical seed mixture available *ad libitum* (but see below), unrestricted access to cuttlebone, water and sand, and were supplemented with 0.42 g of egg food per bird three times per week.

During adulthood, we manipulated aviaries to have either low or high foraging costs (four aviaries each, two per sex, eight aviaries in total) as described in Koetsier and Verhulst (2011). Each aviary was equipped with a food container ($L \times W \times H$: 120 × 10 × 60 cm) with 10 holes in the sides to access food, which was suspended from the aviary ceiling. In the low foraging cost treatment, food containers had perches beneath the holes, whereas in the high foraging cost treatment these perches were removed. Hence, when perches were absent, birds were forced to fly from a distant perch to the food container and back for each seed. Seeds spilt by birds while feeding were collected by a duct, and hence were not accessible for the birds. Birds were exposed to their respective treatment for life, not subjected to other experimental manipulation, and left undisturbed except for occasional blood sampling, weighing and nocturnal respirometry measurements (Briga and Verhulst 2017). The birds were housed with same sex individuals only and hence non-breeding. The experiment was balanced with respect to sex, i.e. there were four aviaries with males and four aviaries with females.

Mass and size

Body mass was measured monthly for all the birds and the measurement closest to the blood sampling session (mean ± SEM = 2.9 ± 0.1 days between blood sample and mass measurement) was taken for statistical analyses (birds were not weighed at sampling to minimize potential effects of prolonged handling on baseline glucose). Note that body mass is highly repeatable in our experimental population (Briga and Verhulst 2017). Mass variation between individuals can be due to variation in size, and in other factors such as energy reserves, and to remove the size effect we calculated residual body mass from a regression of body mass on structural size. Structural size was measured when birds reached 100 days old and corresponds to a combined measurement of the tarsus and the head-bill length, both standard normally distributed using the equation: (standardized tarsus + standardized head-bill)/2.

Glucose

Baseline glucose was defined as the stable glucose concentration at rest and at least 30 min after the last meal. For measuring baseline glucose, blood sampling was carried out on the same experimental population in two periods: (1) July 1st–August 9th, 2012, and (2) September 21st–November 3rd, 2014. Blood sampling was done between 9:00 and 18:00 h. Sample size was 171 birds (0.4–6.6 years old; mean ± SEM 3.1 ± 0.16 years) in the first sampling period (2012), and 135 birds (0.9–8.3 years old; mean ± SEM 3.4 ± 0.17 years) in the second sampling period (2014). To quantify the repeatability of baseline glucose, 57 birds were sampled twice within the same year (29 birds in 2012 and 28 birds in 2014) and 81 from the 171 birds sampled in 2012 were re-sampled in 2014. The same sampling protocol was followed in all sampling periods (see below).

Temperature and day length may affect baseline glucose because energy turnover is likely to be lower on long and warm days. Furthermore, controlling for these factors may increase statistical power when testing for treatment effects. We estimated temperature using data loggers installed inside the aviaries, and day length (time between sunrise and sunset) using the information available online https://www.timeanddate.com/sun/netherlands/groningen.

Before sampling, birds were removed from their aviary and individually housed in a small ($L \times W \times H$: 40 × 40 × 15 cm) box without access to food or water. The box was placed, for 30 min, in a dark room (to maintain activity levels low and hence more or less constant across individuals), at the same ambient temperature as the aviary, together with two other boxes containing birds from the same aviary. The aim of this procedure was to yield baseline glucose values independent of recent food consumption. A pilot study, conducted using this method on a different sample of birds of the same colony, showed that intra-individual baseline glucose was stable between 30 and 60 min after the capture: No difference was found in glucose level between 0 and 30 min after catching (paired $t = -1.89$, $df = 25$, $P = 0.08$), between 30 and 60 min after catching (paired $t = -0.25$, $df = 25$, $P = 0.81$), or between 45 and 60 min after catching (paired $t = -0.84$, $df = 15$, $P = 0.41$). However, baseline glucose levels increased between 60 and 75 min after catching (paired $t = -4.05$, $df = 15$, $P = 0.001$). Consequently, to minimize the duration of the procedure and the associated stress we used the minimum waiting time within this interval (i.e. 30 min.), after which 70 µL blood sample was taken from the brachial vein and collected in heparinized capillaries. Immediately after sampling, blood was diluted 30x in a heparin (500 IU/mL) − 0.01% EDTA solution and

frozen at − 20 °C for a maximum of 48 h, before glucose levels were measured. Laboratory measurements were performed within the same year that samples were obtained, and calibration curves were included in each assay.

Baseline glucose levels were measured using the Hoffman's ferricyanide method and a Thechnicon autoanalyzer (Beckman Coulter LX20PRO). For internal validation, autoanalyzer readings were scaled to a standard glucose curve included at the beginning and end of each batch of measurements. Blood samples taken in 2014 (135 birds included in the analyses reported here and 27 additional birds from a pilot study) were analyzed in duplicates obtaining an intraclass correlation coefficient (ICC) of 72.4% ($n = 324$ measurements, 95% CI 64.5, 79%). Statistical calculations were done on the average of the two duplicates. In 2012 measurements were not done in duplicates.

Statistical analyses

All statistical analyses were performed using R version 3.4.2 (R core team 2017). Glucose values were ln transformed prior to analysis to obtain a normal distribution. Because we were primarily interested in within-year variation, we used mean-centered baseline glucose per year as dependent variable in all analyses. Linear mixed models were fitted using the function lmer of the package lme4 (Bates et al. 2015). In all fitted models, repeated measures per individual were accounted for by including individual identity as a random factor.

We used stepwise backward deletion of non-significant terms in all the analyses. We first evaluated the role of physical environment of year of sampling, time of the day, time of the day squared, day length and ambient temperature on glucose levels. In the second analysis, we tested the effect of sex, age, age squared and residual body mass (from regression of mass on size). Note that in our study we can only test for a cross-sectional association between age and glucose levels, and such tests yield biased results when there is selective disappearance with respect to glucose level. A longitudinal analysis can resolve this issue, but in our study year and age difference are confounded, because we have only two sampling years. Finally, we then evaluated the effect of experimental treatments (rearing brood size, foraging treatment and their interaction) on baseline glucose, including bird identity and sampling date as random terms.

To test for the association between glucose and survival, we fitted Cox proportional hazards (CPH) models (coxme package; Therneau 2012). Models included glucose, glucose squared and the experimental treatments. To avoid regression to the mean, we used only the first annual sample of each individual. To avoid the confounding effects of day and temperature on survival (see Briga and Verhulst 2015), we used the glucose residuals from the linear regression on Julian day and ambient temperature. CPH analyses require predictors to be proportional, i.e. parameter coefficients need to be constant with time (Therneau and Grambsch 2000, p. 127). All survival analyses were checked for the proportionality assumption using Schoenfeld residuals and with the 'cox.zph' function. However, the effect of sampling age on mortality accelerated with time (adding 1 year of sampling age has a stronger effect on the mortality of old than of young birds) and hence was not proportional. We solved this issue by categorizing sampling age in two approximately equally sized groups, splitting at the mean age at sampling of 3.3 years and included this as "strata" in the models (Therneau and Grambsch 2000, p. 45 and 145). Linearity and influential data points were checked with Martingale and deviance residuals, respectively.

Results

Repeatability

We calculated the repeatability of baseline glucose on two levels: within years and between years. Data for the repeatability estimates were collected in two sessions in each of the 2 years (i.e. four sessions in total). Because average baseline glucose differed between these four sessions ($P < 0.0001$), and because we were primarily interested in the consistency of individual differences, we used deviations from the session average to calculate repeatability adjusted for session effects. Within-year repeatability (intra-class correlation coefficient) was 29.7% (Fig. 1a, $n = 57$ individuals, 95% CI 2.7–56.7%). Similarly, between-years repeatability was 27.4% (Fig. 1b, $n = 81$ individuals, 95% CI 4.7–50.1%; using only the first glucose measurement per year). Thus, individual differences in baseline glucose are repeatable, even over a period of years.

Physical environment

Baseline glucose was almost 20% lower during the first of the two study years (Fig. 2a; $F_{1,229.51} = 33.18$, $P < 0.001$; first year mean ± SEM 12.98 ± 0.10 mM; second year: 16.03 ± 0.18 mM). To ensure that our analyses examined within rather than between year effects, we mean centered glucose measurements per year in the following sections. Baseline glucose was lower at higher temperatures and on longer days (Table 1; Fig. 2). Considering these results, and to increase statistical power when testing for effects of individual characteristics, we included sampling day as a random factor in subsequent analyses. This factor accounts for environment fluctuation associated with temperature, day

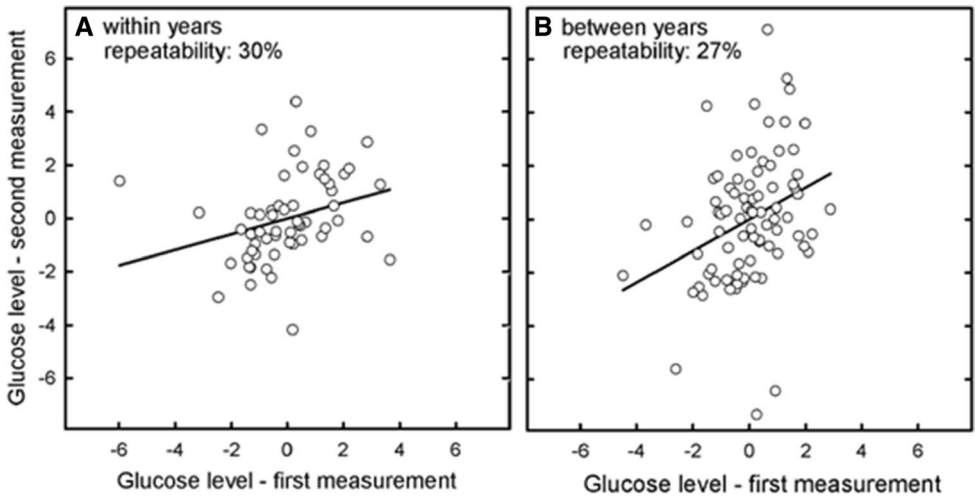

Fig. 1 Individual repeatability of baseline glucose levels (mM) within years (**a**) and between years (**b**). Plotted data show second measurement plotted against the first measurement in both cases. Data points are deviations from the mean glucose level in each of the measurement sessions to account for within and between year variations in average glucose level. Intra-class correlation coefficient, within years $n = 57$ individuals, 95% CI 2.7–56.7%, between years $n = 81$ individuals, 95% CI 4.7–50.1%

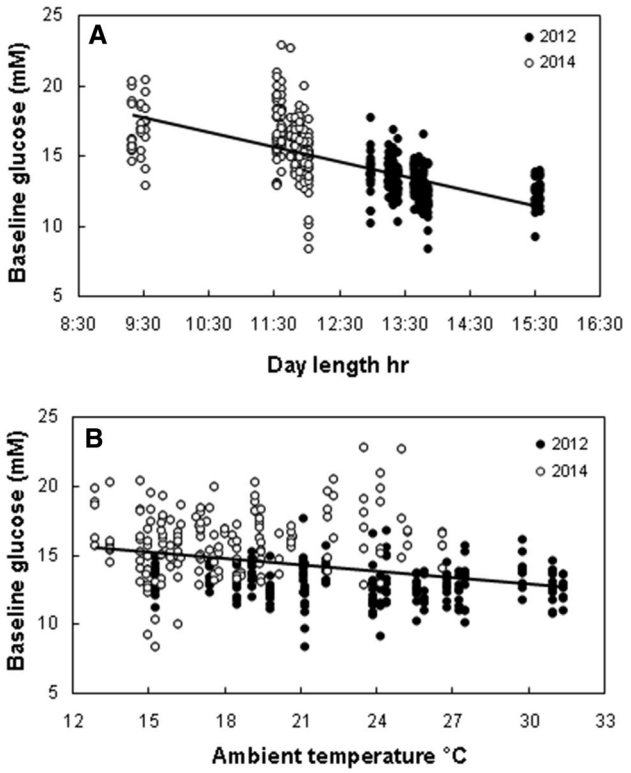

Fig. 2 Baseline glucose level (mM) in relation to day length (**a**) and ambient temperature (**b**). Closed circles correspond to birds sampled in 2012 and open circles to birds sampled in 2014. General linear mixed model, $n = 363$ samples collected on 225 individuals (Day length $F_{1,262.87} = 42.81$, $P < 0.001$; ambient temperature $F_{1,329.77} = 6.71$, $P = 0.01$, individual identity was included as a random factor to control for repeated measurements)

length and other potentially unidentified sources of variation among sampling days. Time of the day at which an individual was sampled did not affect baseline glucose (Table 1).

Sex, age, size and body mass

Sex did not explain a significant portion of the variation on baseline glucose ($F_{1,280.51} = 0.01$, $P = 0.95$), and was therefore excluded from subsequent models.

Basal metabolic rate declines with age in our study species (e.g. Moe et al. 2009), also in our study population (Briga 2016). However, baseline glucose was not associated with age ($F_{1,247.08} = 0.05$, $P = 0.82$), or age squared ($F_{1,179.57} = 0.02$, $P = 0.89$).

There is usually a strong positive association between energy turnover and body mass (for example, Rønning et al. 2007; Briga and Verhulst 2017), but there was no association between baseline glucose and residual body mass ($F_{1,292.87} = 0.25$, $P = 0.61$), body mass or structural size (included in the model one at a time instead of residual body mass; $F_{1,303.19} = 0.39$, $P = 0.53$ and $F_{1,197.87} = 0.23$, $P = 0.63$ respectively). Finally, there was a non-significant trend for sex to interact with residual body mass ($F_{1,289.46} = 3.45$, $P = 0.06$), females with lower, and males with higher, residual body mass tended to have higher baseline glucose, but neither correlation was by itself significant.

Developmental and adult environments

Given that baseline glucose levels were an individual characteristic, i.e. a repeatable trait, we tested whether it was affected by our permanent experimental manipulations, i.e. rearing brood size and adult foraging effort. Baseline glucose was higher in birds reared in large broods, and in birds exposed to high foraging costs while there was no significant interaction between brood size and foraging cost (Table 2; Fig. 3). Individual observations within aviaries are not statistically independent, but adding aviary identity as random

Table 1 Relationship between plasma baseline glucose levels (mM, ln transformed) and with physical environment factors

Fixed effects	Coefficient (±SE)	*df*	*F*	*P*
Temperature	0.004 ± 0.001	1329.77	6.71	0.010
Day length	− 1.15 ± 0.18	1262.87	42.81	< 0.001
Year (relative to 2012)	0.11 ± 0.02	1229.51	33.18	< 0.001
Rejected terms				
Time of the day	− 0.05 ± 0.08	1336.89	0.41	0.52
Time of the day^2	− 0.13 ± 0.54	1291.26	0.059	0.81
Random effects		Variance		
Bird identity		0.0043		
Residual		0.0106		

$n = 363$ samples collected on 225 individuals, 57 individuals were sampled twice within a year and 81 individuals between years. Bird identity LRT = 14.04, $df = 1$, $P < 0.001$

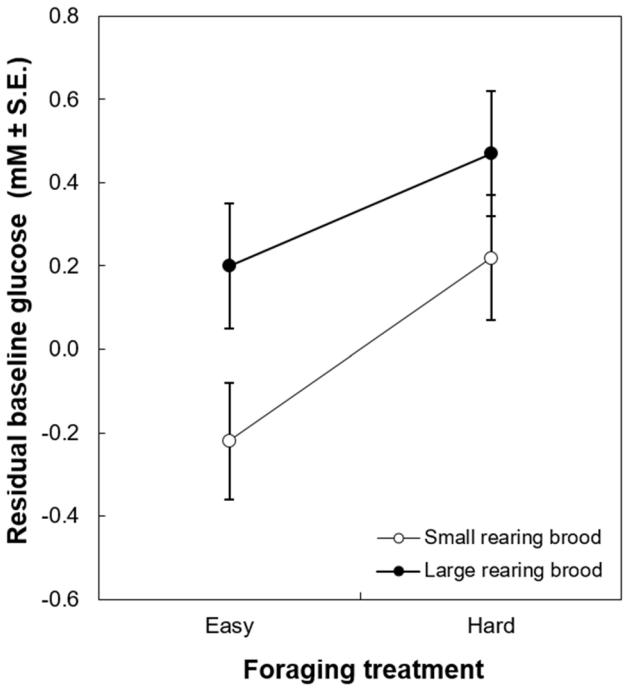

Fig. 3 Manipulated environmental conditions (rearing brood size and foraging treatment) and model estimates of baseline glucose (mM ± SEM; data mean centered by year). Open circles correspond to birds reared in small broods (two chicks), and closed circles correspond to birds reared in large broods (six chicks). General linear mixed model, $n = 363$ samples collected on 225 individuals (Foraging treatment $F_{1,200.34} = 5.58$, $P = 0.02$; rearing brood size $F_{1,206.88} = 4.50$, $P = 0.04$, individual identity was included as a random factor to control for repeated measurements)

effect to the model did neither explain significant variation (LRT, $P = 0.22$), nor cause substantial changes in the model. Baseline glucose repeatability adjusted for treatment effects was 29% (95% CI 13.2–43.5%).

Survival

By January 2016, 120 of the 171 birds sampled in 2012 and 56 of the 135 birds sampled in 2014 had died. Note that some birds were sampled in both years, and of the 225 birds sampled in total, 176 had died. The cause of natural death was rarely if ever clear. Birds with the highest baseline glucose had lower survival probability (Fig. 4). This pattern was very similar in the two sampling years (Fig. S1), and independent of sex (glucose × sex interaction: $z = -0.35$, $P = 0.72$). This result did not change when we controlled for the two experimental manipulations and their interaction (Table 3), or the manipulations without the interaction (data not shown). We also tested for a quadratic association between survival and baseline glucose, by adding baseline glucose level squared to the model, but this did not improve the model fit ($\beta = -0.002 \pm 0.012$, $P = 0.88$).

Discussion

Individual variation in baseline glucose was significantly repeatable, and repeatability within and between years was almost indistinguishable, at 30 and 27%, respectively. Repeatability remained at this level (29%) when it was adjusted for the treatment effects. We did not find estimates of baseline glucose repeatability in the literature for comparison, but our estimates are close to the repeatability value of 32% found on average for physiological traits (Wolak et al. 2012). Furthermore, our estimate falls within the range reported for other physiological traits in zebra finches, 18–46% (Rønning et al. 2005; Careau et al. 2014), also in our study population (Jimeno et al. 2017b; Briga and Verhulst 2017). Thus, individual zebra finches can be characterized by their baseline glucose.

Baseline glucose levels depended on current and historical environmental conditions. With respect to current

Table 2 Relationship between glucose levels (mM, mean-centered by year) and experimental treatments

Variable (fixed effect)	Coefficient (±SE)	*df*	*F*	*P*
Foraging treatment (high cost)	0.24 ± 0.10	1208.32	5.21	0.02
Rearing brood size (large brood)	0.22 ± 0.11	1211.41	4.23	0.04
Rejected terms				
Foraging treatment × rearing brood size	0.04 ± 0.21	1207.81	0.04	0.84
Random effects		Variance		
Bird identity		0.2678		
Sampling date		0.1860		
Residual		0.4940		

$n = 363$ samples collected on 225 individuals, 57 individuals were sampled twice within a year and 81 individuals between years. Bird identity and sampling day LRT = 13.09, $df = 2$, $P < 0.001$. Baseline glucose differed significantly between years, even after controlling for variation of the physical environment (i.e. temperature, day length; Table 1), then baseline glucose values were mean-centered by year (i.e. the mean value of the sampling year was subtracted from each observed glucose value). Sampling day was included as random term to control for within year variation in the physical environment

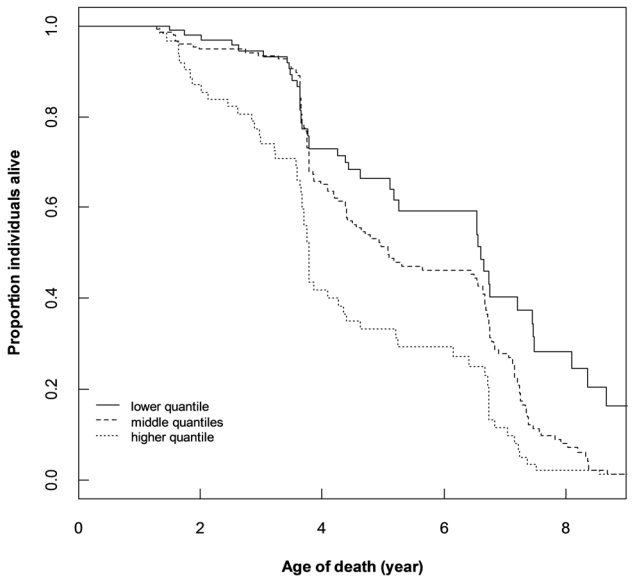

Fig. 4 Plasma baseline glucose level and survival. The solid line corresponds to low glucose level (mM, lower quartile), dashed line to intermediate glucose (two middle quartiles pooled), and dotted line to high glucose (upper quartile). Note that this grouping is for illustrative purposes only; glucose level was treated as a continuous variable in the analysis and years were pooled using glucose levels mean centered by year. Cox proportional hazards model, $n = 304$ samples collected on 225 individuals of which 176 died (residual glucose $z = 2.37$, $P = 0.02$; see also Table 3)

environmental conditions, baseline glucose was lower in the low foraging costs treatment, with higher ambient temperatures and on longer days (i.e. longer light period). Such associations have previously been reported for various bird and mammal species (Bairlein 1983; Remage-Healey and Romero 2000; Schradin et al. 2015). Higher ambient temperature and low foraging costs are both likely to result in lower diurnal energy expenditure (Wiersma and Verhulst 2005; Koetsier and Verhulst 2011). These findings, therefore, indicate that baseline glucose is regulated at a lower level when energy turnover is low, but direct measurements are required to verify this inference. The negative association between day length and glucose level can also be interpreted from this perspective, because on long days the birds have more hours to fulfill their energy needs which presumably will be reflected in a lower rate of energy turnover (Kersten and Piersma 1987). Age did not explain variation in baseline glucose, despite the fact that nocturnal energy turnover of zebra finches has been shown to decline with age (Moe et al. 2009). However, to what extent age affects energy turnover of zebra finches during the active phase is not yet known, and animals have ample leeway for energetic compensation during the active phase, e.g. by altering activity pattern. Hence, daytime energy expenditure in the active phase may well be independent of age in our experiment. The suggestion emerging from our study that glucose levels are positively associated with energy expenditure may also explain the observation that birds have higher glucose levels than mammals, because birds have higher energy expenditure per unit mass than mammals (Speakman 2005). Likewise, it may explain why glucose levels, in birds, decrease with mass across species (Braun and Sweazea 2008), because mass specific energy expenditure decreases with increasing body mass in birds and mammals (Speakman 2005).

In addition to effects of current environmental conditions, baseline glucose also depended on historical environmental conditions, with birds reared in large broods having 11% higher baseline glucose than birds raised in small broods (Fig. 3). This finding is consistent with the proposed long-term effects of early-life nutritional restriction on glucose homeostasis in mammals (Desai et al. 1996; Gardner et al. 2005; Fagundes et al. 2007). However, whether early nutritional restriction affects glucose regulation through similar

Table 3 Relation between residual glucose (mM) and mortality probability, fitted with a Cox proportional hazards model

Fixed effects	Exp (coef.) ± SE	z	P
Residual glucose	1.09 ± 0.04	2.28	0.02
Sex	0.49 ± 0.18	− 3.73	< 0.001
Rearing brood size (large brood)	0.96 ± 0.05	− 0.64	0.52
Foraging treatment (high cost)	1.30 ± 0.35	0.75	0.46
Rearing brood size × foraging treatment	0.99 ± 0.78	− 0.08	0.94
Random effects		Variance	
(Bird identity) Aviary		0.02	

For the cox proportional hazards model, residual glucose was calculated from the linear regression of sampling date and ambient temperature on baseline glucose mean centered by year. n = 304 samples collected on 225 individuals of which 176 died in the study period (only the first sample of each individual in a sampling year was included in the survival analysis)

mechanisms in birds as in mammals remains to be investigated. The effect of being reared in a large brood was independent of the current foraging costs, because early and adult life manipulations did not significantly interact to affect baseline glucose in adulthood (Table 2). The absence of this interaction contrasts with predictions following from the thrifty phenotype hypothesis, according to which one would expect the response to nutritional stress in adulthood to depend on the nutritional stress during development following a match-mismatch pattern (Hales and Baker 1992, 2001; Gluckman et al. 2005; Hanson and Gluckman 2014). However, our finding is in agreement with the lack of broad support for the match–mismatch hypothesis emerging from a recent meta-analysis, summarizing effects on a wide range of traits, including physiology, reproduction and survival, in animals and plants (Uller et al. 2013).

High baseline glucose was associated with higher mortality (Fig. 4). We are not aware of previous studies testing for this relationship in species other than humans, except that Récapet et al. (2016) observed higher mortality in collared flycatchers with higher glycosylated hemoglobin, which presumably reflects long-term high glucose levels. Our study differs from the studies on humans and collared flycatchers in that diet was standardized, and hence diet is unlikely to be a confounding of individual variation in baseline glucose. Long-term elevated glucose is considered a form of metabolic stress and has been proposed to produce mitochondrial dysfunction and mitochondrial DNA damage, promoting inflammation, altering gene expression and accelerating ageing (Picard et al. 2014). Mitochondrial dysfunction may affect organism's adaptive capacity, through widespread mitochondrial fragmentation that impairs energy production (Morava and Kozicz 2013; Picard et al. 2014), which may underline the deteriorated survival probability of individuals with high baseline glucose in our study.

We previously found in the same experimental population that birds reared in large broods and exposed to high foraging costs achieved a shorter lifespan than birds relative to all other treatment combinations (Briga et al. 2017). One of our aims in studying physiological variation was to identify physiological processes that vary in parallel with the observed experimental effects on lifespan. However, the treatments and their interaction did not significantly affect survival in the present data set (Table 3; removing baseline glucose from the model does not change this result). This contrast with our findings in the complete data set (Briga et al. 2017) is not unexpected however, because the experimental survival effects were most conspicuous in early adulthood, and birds in the present data set were generally older because the experiment had been running for 4 years when the first data for the present paper were collected. Thus, to what extent the experimental survival effects can be attributed to processes reflected in baseline glucose cannot be inferred from the present data set. Instead, we can conclude that baseline glucose is associated with mortality over and above mortality effects of the environmental manipulations.

An increase in baseline glucose with age has previously been reported in other bird species (Ferrer and Dobado-Berrios 1998; Prinzinger and Misovic 2010), but despite a substantially larger sample size we found age to be unrelated to baseline glucose in our cross-sectional analysis. Selective disappearance of individuals with high baseline glucose would have generated a negative cross-sectional association between age and baseline glucose when glucose levels do not change with age, and there was no evidence for such relationship. Therefore, the positive relationship between baseline glucose and mortality in combination with the absence of a cross-sectional age effect suggests that glucose levels increase with age within-individuals. A longitudinal study is required to test this interpretation.

Our finding that elevated baseline glucose is associated with increased mortality raises the question why individuals regulate their baseline glucose at high levels when exposed to harsh environments. We find blood glucose levels to be positively associated with proxies of energy expenditure, which from a functional perspective is in agreement with

the finding that high baseline glucose was found to enhance performance during physically and cognitively demanding activities (Rodríguez et al. 2009; Gilsenan et al. 2009). Thus, elevated glucose levels can possibly be explained from a functional perspective by the short-term benefits this yields when encountering a harsh environment, when these short-benefits outweigh the long-term survival cost associated with higher baseline glucose. The finding that glycosylated hemoglobin levels, a variable that integrates blood glucose levels over a longer period, correlated positively with reproduction and negatively with survival in collared flycatchers (Andersson and Gustafsson 1995; Récapet et al. 2016) is in agreement with this hypothesis. Therefore, baseline glucose may more generally reflect individual position in the slow-fast life-history continuum, with high glucose levels facilitating short-term fitness benefits at the expense of future fitness benefits.

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Author contributions SM performed bird sampling and glucose measurements in 2012, and BM and BJ in 2014. BM, MB and SV analyzed data. MB and BJ ran the long-term experiment designed by SV, and collected survival, body mass and structural size data. BM, SV and MB wrote the first draft of the manuscript and all authors contributed to later versions.

Compliance with ethical standards

Data accessibility All data used in this manuscript will be made available upon acceptance.

Conflict of interest The authors declare no competing interests.

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